PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C07K 14/47, C12N 15/12, C07K 16/18, C12N 15/70, G01N 33/68, C12Q 1/68, A61K 38/17, C07K 19/00

A1

(11) International Publication Number: **WO 96/23000**

(43) International Publication Date: 1 August 1996 (01.08.96)

(21) International Application Number: PCT/US96/01079

(22) International Filing Date: 16 January 1996 (16.01.96)

(30) Priority Data:
08/379,802 27 January 1995 (27.01.95) US

(71) Applicant: AMGEN INC. [US/US]; Amgen Center, 1840 Dehavilland Drive, Thousand Oaks, CA 91320-1789 (US).

(72) Inventors: BARTLEY, Timothy, D.; 2431 McCrea Road, Thousand Oaks, CA 91362 (US). FOX, Gary, M.; 35 West Kelly Road, Newbury Park, CA 91320 (US).

(74) Agents: ODRE, Steven, M. et al.; Amgen Inc., Amgen Center, 1840 Dehavilland Drive, Thousand Oaks, CA 91320-1789 (US).

(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AZ, BY, KG, KZ, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: LIGANDS FOR EPH-LIKE RECEPTORS

(57) Abstract

Polypeptides which bind to one or more EPH-like receptors, particularly the HEK4 receptor, are described. The polypeptides are designated HEK4 binding proteins. Nucleic acids encoding HEK4 binding proteins, and expression vectors, host cells and processes for the production of the polypeptides are also described. The polypeptides are useful for modulating the growth and/or differentiation of a variety of tissues, including those from liver, kidney, lung, skin, digestive tract and nervous system and may be used to regenerate damaged or depleted tissue and to treat cancer or nervous system disorders.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgystan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic			SE	Sweden
CG	Congo	KR	Republic of Korea	SG	Singapore
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LR	Liberia	SZ	Swaziland
CS	Czechoslovakia	LT	Lithuania	TD	Chad
CZ	Czech Republic	LU	Luxembourg	TG	Togo
DE	Germany	LV	Latvia	TJ	Tajikistan
DK	Denmark	MC	Monaco	TT	Trinidad and Tobago
EE	Estonia	MD	Republic of Moldova	UA	Ukraine
ES	Spain	MG	Madagascar	UG	Uganda
FI	Finland	ML	Mali	US	United States of America
FR	France	MN	Mongolia	UZ	Uzbekistan
GA	Gabon	MR	Mauritania	VN	Viet Nam

LIGANDS FOR EPH-LIKE RECEPTORS

The invention relates to polypeptides which bind to one or more EPH-like receptors. More particularly, the invention relates to polypeptides which bind to the HEK4 receptor, to nucleic acids encoding same and to expression vectors and host cells for the production of the polypeptides.

Background of the Invention

The response of cells to their environment is often mediated by soluble protein growth and differentiation factors. These factors exert their effects by binding to and activating transmembrane receptors. This interaction is the initial event in a cascade which culminates in a biological response by the cell. An important class of transmembrane receptors is the receptor protein tyrosine kinases (receptor PTKs, reviewed in van der Geer et al. Ann. Rev. Cell. Biol. 10, 251-337 (1994). PTKs consist of an extracellular domain which interacts specifically with the receptor's cognate ligand, a membrane spanning domain, and an intracellular domain which harbors the tyrosine kinase activity. Receptor PTKs are activated by ligand-mediated dimerization followed by autophosphorylation of tyrosine residues in the cytoplasmic domain. The receptor PTK can then in turn phosphorylate substrate molecules in the signal transduction pathway, leading to a cellular response.

The family of receptor PTKs can be divided into a number of sub-families based on the general structure of the extracellular domain and on amino acid sequence relationships within the catalytic domain. Currently, the largest known sub-family of

receptor protein tyrosine kinases is the EPH-like receptors, consisting of at least 13 members. Members of this sub-family include the following: EPH (Hirai et al., Science 238, 1717-1725 (1987)), ECK (Lindberg et al., Mol. Cell. Biol. 10, 6316-6324 (1990)), Cek4, Cek5, Cek6, Cek7, Cek8, Cek9, Cek10 (Pasquale, Cell Regulation 2, 523-534 (1991); Sajjadi et al., The New Biologist 3, 769-778 (1991); Sajjadi and Pasquale , Oncogene 8, 1807-1813 (1993)), Eek, Erk (Chan and Watt, Oncogene 6, 1057-1061 (1991)), Ehk1, Ehk2 (Maisonpierre et al., Oncogene 8, 3277-3288 (1993)), HEK (PCT Application No. WO93/00425; Wicks et al., PNAS 89, 1611-1615 (1992)), HEK2 (Bohme et al., Oncogene 8, 2857-2862 (1993)), HEK5, HEK7, HEK8, HEK11 (U.S. Serial No. 08/229,509) and HTK (Bennett et al. J. Biol. Chem. 269, 14211-14218 (1994)).

Until recently, no ligands for any member of the EPH sub-family had been identified. A ligand for the Eck receptor was described in PCT Application No. WO 94/11020 and Bartley et al. (Nature 368, 558-560 (1994)) and identified earlier as B61, a polypeptide encoded by a cDNA of unknown function (Holzman et al., Mol. Cell Biol. 10, 5830-5838 (1990)). Ligands for Elk and Ehk1 receptors have also been reported (PCT Application No. WO94/11384; Davis et al., Science 266, 816-819 (1994)). Most recently, a polypeptide (ELF-1) identified from a mouse embryo midbrain and hindbrain cDNA library has been reported to be a ligand for Mek4 and Sek (Cheng and Flanagan, Cell 79, 157-168 (1994).

Most attempts to purify soluble factors from complex biological fluids have depended on cell-based bioassays of the response to stimulation by the factor. These include increased cell growth or survival, increased DNA synthesis, a chemotactic response, or some other downstream consequence of

receptor activation. Receptor autophosphorylation has also been used as an assay to detect stimulation of the cell. We have recently described a method for the isolation of ligands based on direct detection of receptor/ligand binding and the use of receptor affinity chromatography for purification (Bartley et al., supra). Here we report the application of this method to purify, sequence, and molecularly clone one of a family of ligands corresponding to the EPH sub-family of receptor tyrosine kinases.

Although the EPH sub-family is the largest known sub-family of receptor PTKs, few ligands have been identified which bind to and activate an EPH sub-family receptor. It is therefore an objective to identify additional ligands for EPH sub-family receptor PTKs. These ligands will be useful for modulating responses of EPH sub-family receptor bearing cells.

Summary of the Invention

The present invention relates to polypeptides capable of binding to one or more EPH-like receptor PTKs. More particularly, the invention provides polypeptides which bind to the HEK4 receptor, but may also bind to other members of the sub-family of EPH-like receptor PTKs. These polypeptides are referred to as HEK4 binding proteins (HEK4 BPs). In one embodiment, the polypeptide binds to and activates HEK4 and ECK receptors. Also encompassed by the invention are nucleic acids encoding HEK4 BPs and nucleic acids which hybridize to HEK4 BP nucleic acids and encode polypeptides having at least one of the biological properties of a HEK4 BP. Biologically active HEK4 BP fragments and analogs and nucleic acids

encoding same as well as fusion proteins comprising HEK4 BP are also encompassed by the invention.

Expression vectors and host cells for the production of biologically active HEK4 BP and processes for the production of HEK4 BP using the expression vectors and host cells are also within the scope of the invention. Antibodies specifically binding HEK4 BP are also provided for.

Polypeptides of the invention are useful for modulating (i.e,. increasing or decreasing) the growth and/or differentiation of EPH sub-family receptor-bearing cells, particularly cells expressing HEK4 or ECK receptors. Based on levels of expression of HEK4, ECK, and HEK4 BP in a variety of tissues, it is expected that HEK4 BP will be useful for modulating the growth and/or differentiation, for example, liver, kidney, lung, skin or neural tissues. Administration of HEK4 BP to mammals is useful in the treatment of nervous system disorders and in the regeneration of damaged or depleted tissues. HEK4 BP antagonists are also useful for the treatment of cancers.

Description of the Figures

Figure 1. BIAcore screening of conditioned media on HEK4-X surface. Concentrated samples of cell-conditioned media were screened on a HEK4-X surface as described in Example 2. The number of conditioned media samples giving a signal within each range of resonance units (RU) are shown in the histogram. Samples which bound more than 200 RU are summarized in Table 1.

Figure 2. Purification of HEK4 Binding Protein from A498 conditioned media. C4 Reverse Phase

HPLC column profile of HEK4 BP (a); SDS-PAGE analysis of pools of indicated peaks observed on the C4 column.

Figure 3. Sequence of HEK-4 binding protein cDNA. The nucleic acid sequence of the human HEK-4 binding protein cDNA clone containing the entire coding sequence is shown along with the predicted amino acid sequence. The cDNA clone predicts a protein of between 213 and 228 amino acids, depending on which of three potential start codons is utilized. The sequence is numbered so that the predicted mature N-terminal amino acid is residue 1, with the putative signal peptide (underlined) extending from residues -19 to -1.

Figure 4. Purification of recombinant HEK-4 binding protein. C4 Reverse Phase HPLC column profile of recombinant HEK4 BP (a); SDS-PAGE analysis of C4 fractions in the vicinity of the A_{214} peak. Fractions are identified by elution times from the C4 column.

Figure 5. Expression of HEK-4 binding protein in human tissues. The expression of HEK-4 binding protein mRNA in human tissues was examined by Northern blot analysis as described in Example 6. A blot containing 2 μg of polyA+ mRNA isolated from each of several tissues was purchased from Clontech (Palo Alto, CA) and hybridized with a ^{32}P-labeled HEK-4 binding protein cDNA probe.

Figure 6. Stimulation of tyrosine phosphorylation of EPH-like receptors by membrane-bound HEK4 BP. CHO cells that express recombinant HEK4 receptor and endogenous ECK were treated with cells that were transfected with an

expression vector that contained the HEK4 BP cDNA or vector without cDNA. After lysis, HEK4 receptor (a) or ECK receptor (b) were immunoprecipitated. The immunoprecipitates were fractionated by PAGE, electroblotted, and probed with antiphosphotyrosine antibodies.

Figure 7. Stimulatation of tyrosine phosphorylation by soluble HEK4 BP. Cells were treated with conditioned media (CM) or recombinant HEK4 BP, with (+) or without (-) antibody clustering, and assayed for HEK4 receptor activation. a) Twelve-fold concentrated media was compared to 2µg/ml HEK4 BP. (b) A dose response comparing clustered and unclustered HEK4 BP.

Figure 8. Relative affinity of HEK4 BP for HEK 4, ECK and HEK8 receptors. A competition assay for measuring binding of HEK4 BP to immobilized HEK4 receptor in the presence of increasing concentrations of soluble HEK4, ECK and HEK8 receptors was performed as described in Example 8. The line identified as "IC 50" is drawn at an RU value corresponding to a HEK4 BP concentration which is 50% of the control (no competitor) RU value.

Detailed Description of the Invention

The present invention relates to polypeptides capable of binding to one or more EPH-like receptor PTKs and, more particularly, are capable of binding to a human homolog of an EPH-like receptor PTK. To date, eight human homologs of EPH-like receptors have been identified: EPH, ECK, HTK, HEK2, HEK4, HEK5, HEK7, HEK8 and HEK11. Characteristics of several HEK receptors are disclosed

in co-pending and commonly owned U.S. Serial No. 08/229,509 herein incorporated by reference. The polypeptides of the present invention preferentially bind HEK4 receptor and are referred to herein as HEK4 binding proteins (HEK4 BP). The HEK4 receptor is a glycosylated 135 kDa protein tyrosine kinase previously identified as HEK receptor by Wilks et al. supra, and is the human homolog of the Cek 4 and Mek 4 receptors identified in chicken and mouse, respectively. Polypeptides capable of binding HEK4 receptor may also activate the receptor by inducing receptor autophosphorylation, an event which initiates transmission of a signal from the cell surface to the nucleus. Activation of HEK4 receptors leads to modulation of growth and/or differentiation of HEK4 receptor-bearing cells. HEK4 BP also binds to and activates other EPH-like receptors as described below.

A HEK4 binding protein has been identified and isolated from A498 cell line conditioned medium by procedures generally described in U.S. Serial No. 08/145,616, relevant portions of which are herein incorporated by reference, and described in more detail in Example 2. Briefly, a gene encoding the extracellular domain of the HEK4 was constructed and expressed as described in Example 1. The purified HEK4 extracellular domain was immobilized on a BIAcore sensor chip and concentrated conditioned media from 102 different cell lines were screened for binding to HEK4 receptor extracellular domains by surface plasmon resonance. This procedure identified conditioned medium from several cell lines shown in Table 1 having one or more factors which interacted with HEK4. The A498 cell line was chosen as a source for HEK4 ligand and a protein binding to HEK4 was purified as described in Example 2. Purified and isolated HEK4

binding protein from A498 cell-conditioned medium has three major forms of molecular weights 21, 25 and 27kD on nonreducing SDS-polyacrylamide gels. These forms represent glycosylation and C- terminal processing variants. HEK4 BP has the amino acid sequences as shown in Table 2 for the peptides generated by cyanogen bromide or trypsin cleavage.

cDNA clones of HEK4 binding protein were obtained from a human placenta cDNA library as described in Example 3. The sequence of human HEK4 binding protein cDNA is shown in Figure 3. Based upon cDNA sequencing and carboxy-terminal peptide mapping of the A498 cell-derived protein, a major secreted form of HEK4 binding protein had an amino terminal serine residue as shown in Figure 3 and a carboxy terminal proline residue at position 179. An alternate secreted form having a carboxy terminal alanine residue at position 177 was also detected. Alternative forms of HEK4 BP, including membrane-bound forms, may also be synthesized.

Recombinant HEK4 binding protein was expressed in CHO cells transfected with cDNA encoding HEK4 BP as shown in Figure 3. Soluble HEK4 BP was purified as described in Example 4 and shown to bind HEK4 receptor by BIAcore analysis. Purified soluble HEK4 BP activated HEK4 receptor and this activation was enhanced by antibody clustering of the ligand (Figure 7). Activation of HEK4 receptor was also observed upon contact of CHO cells expressing HEK4 BP with CHO cells expressing HEK4 receptor (Figure 6). Therefore, recombinant HEK4 binding protein binds and activates EPH-like receptor PTKs.

The invention provides for a purified and isolated polypeptide, termed HEK4 binding protein,

capable of binding at least one EPH-like receptor. In one embodiment, the polypeptide is capable of binding the HEK4 receptor. HEK4 binding protein is mammalian and is preferably human. Purified HEK4 binding protein is substantially free of other human proteins and has a molecular weight of about 21 to 27kD on nonreducing SDS-PAGE. HEK4 BP has at least about 70% homology to the amino acid sequence as shown in Figure 3 (SEQ ID NO: 1) and is capable of binding at least one EPH-like receptor. Preferably, HEK4 BP has the amino acid sequence as shown in Figure 3 (SEQ ID NO: 1). Binding of an EPH-like receptor by HEK4 BP may or may not result in receptor activation. EPH-like receptor binding and activation may be effected by either soluble or membrane-bound form of HEK4 BP, or by both forms. It is further understood that receptor binding and activation by HEK4 BP is not restricted to HEK4 receptor, but that HEK4 BP may also bind to and activate other EPH-like receptor family members. As described in Example 7, HEK4 binding protein has been shown to activate both HEK4 and ECK receptors.

HEK4 binding proteins of the invention are preferably characterized by being the product of procaryotic or eucaryotic expression of an exogenous DNA sequence, i.e., HEK4 binding protein is a recombinant protein. Exogenous DNA is DNA which encodes HEK4 binding protein and includes cDNA, genomic DNA and synthetic (manufactured) DNA. HEK4 binding protein may be expressed in bacterial, yeast, plant, insect or mammalian cells in culture or in transgenic animals using DNA expression vectors appropriate for the given host cell. Expression of recombinant HEK4 binding protein in CHO cells is described in Example 3 of the specification.

Also provided is HEK4 BP in dimeric or higher order oligomeric states wherein multimeric

HEK4 BP is capable of binding and/or activating EPH-like receptors. Soluble HEK4 BP multimers are selected from the group consisting of HEK4 BP/immunoglobulin chimeras, HEK4 BP clustered by treatment with anti-HEK4 BP antibodies, and covalently and noncovalently attached HEK4 BP monomers. Clustered HEK4 BP is described in Example 7B and HEK4 BP chimeras are constructed using standard recombinant DNA techniques. Covalently and noncovalently attached HEK4 BP monomers are produced using protein crosslinking reagents and procedures readily available to one skilled in the art.

The polypeptides of the present invention include biologically active fragments and analogs of HEK4 BP. HEK4 BP fragments encompass amino acid sequences having truncations of one or more amino acids from the sequence shown in Figure 3 or in SEQ ID NO: 1, wherein the truncation may originate from the amino terminus, carboxy terminus, or from the interior of the protein. Analogs of the invention involve an insertion or a substitution of one or more amino acids within the sequence as shown in Figure 3 or SEQ ID NO: 1. Fragments and analogs will have at least one biological property of HEK4 binding protein, typically the ability to bind at least one EPH-like receptor.

Also encompassed by the invention are chimeric polypeptides comprising HEK4 BP amino acid sequences fused to heterologous amino acid sequences. Said heterologous sequences encompass those which, when formed into a chimera with HEK4 BP, retain one or more biological or immunological properties of HEK4 BP. In one embodiment, a HEK4 BP/immunoglobulin chimeric protein is encompassed wherein chimeric molecules may aggregate to multimeric forms of HEK4 BP for receptor binding and activation. One example is a chimera of HEK4 BP and the Fc region of IgG.

Also provided by the invention is an isolated nucleic acid encoding HEK4 binding protein. The nucleic acid is selected from the group consisting of:

a) the nucleic acid as shown in Figure 3 (SEQ ID NO: 1);

b) nucleic acids which hybridize under conditions of 6XSSC and 65°C with the coding regions as shown in Figure 3 (SEQ ID NO: 1);

c) nucleic acids which are degenerate to the nucleic acids of (a) and (b).

The nucleic acids may be cDNA, genomic DNA or synthetic (manufactured) DNA. It is understood that the hybridization conditions specified herein allow one skilled in the art to estimate the extent of mismatch between a given nucleic acid and a nucleic acid comprising the coding region as shown in Figure 3 (SEQ ID NO: 1) and that such conditions may be varied by changing salt, temperature and/or length of incubation or adding organic solvent at either the washing or hybridization steps and still allow one to obtain an equivalent level of mismatch during hybridization. Therefore, it is envisioned that the nucleic acids of the invention include those which hybridize with the coding regions in Figure 3 under conditions equivalent to those of 6XSSC and 65°C. Nucleic acid sequences encoding HEK 4 binding protein may have an amino terminal leader sequence and a carboxy terminal membrane anchor sequence or alternatively, may have one or both sequences removed. The encoded polypeptides will have at least one of the biological properties of HEK4 BP.

The nucleic acids of the invention will be operatively linked with nucleic acid sequences so as

to express HEK4 binding protein. Sequences required for expression are known to those skilled in the art and include promoters and enhancer sequences for initiation of transcription, transcription termination sites, ribosome binding sites, and sequences directing polypeptide secretion. A general description of nucleic acid sequences which serve to direct expression of exogenous genes is found in Methods in Enzymology v. 185, D.V. Goeddel, ed. Academic Press, Inc. New York (1990). Sequences directing expression of HEK4 binding protein may be homologous or heterologous. A variety of expression vectors may be used to express HEK4 binding protein in procaryotic or eucaryotic cells in culture. One such vector is pDSRα described in PCT Application No. WO90/14363 which was used to express HEK4 BP in CHO cells (see Example 3). In addition, vectors for tissue-specific expression of HEK4 binding protein in transgenic animals and viral-based gene transfer vectors for expression of HEK4 binding protein in human cells in vivo are also available. The nucleic acid coding regions of HEK4 binding protein may be modified by substitution of preferred codons for optimal expression in an appropriate host cell using procedures available to the skilled worker.

Plasmid pDSRα containing the nucleic acid sequence encoding HEK4 BP as shown in Figure 3 has been deposited with the American Type Culture Collection, Rockville, MD on _____, under ATCC Accession No. _____.

A host cell transformed or transfected with nucleic acids encoding HEK4 binding protein are also encompassed by the invention. Any host cell which produces a polypeptide having at least one of the biological properties of a HEK4 BP may be used.

Specific examples include bacterial, yeast, plant, insect or mammalian cells. In addition, HEK4 binding protein may be produced in transgenic animals. Transformed or transfected host cells and transgenic animals are obtained using materials and methods that are routinely available to one skilled in the art. Host cells may contain nucleic acid sequences having the full-length gene for HEK4 binding protein including a leader sequence and a C-terminal membrane anchor sequence (as shown in Figure 3) or, alternatively, may contain nucleic acid sequences lacking one or both of the leader sequence and the C-terminal membrane anchor sequence. In addition, nucleic acid fragments, variants and analogs which encode a polypeptide capable of binding HEK4 receptor may also be resident in host expression systems. Polypeptides of the invention are produced by growing transformed or transfused host cells under suitable nutrient conditions to express HEK4 BP and isolating the results at polypeptides.

Antibodies specifically binding HEK4 binding proteins of the invention are also encompassed. The antibodies can be produced by immunization with full-length (unprocessed) HEK4 binding protein or its mature forms or a fragment thereof. Antibodies may be polyclonal or monoclonal and may be human or murine-derived. Antibodies of the invention may also be recombinant, such as chimeric antibodies having the murine constant regions on the light and heavy chains replaced by human constant region sequences; or complementary determining region (CDR)-grafted antibodies wherein only the CDR is of murine origin and the remainder of the antibody chain has been replaced by human sequences.

The invention also provides for a pharmaceutical composition comprising a therapeutically effective amount of HEK4 binding protein and a pharmaceutically acceptable adjunct. Examples of pharmaceutically acceptable adjuncts include diluents (Tris, acetate or phosphate buffers), carriers (human serum albumin), solubilizers (Tween, polysorbate), preservatives (thimerosol, benzyl alcohol) and anti-oxidants (ascorbic acid). A more extensive survey of components typically found in pharmaceutical compositions appears in Remington's Pharmaceutical Sciences 18th ed. A.R. Gennaro, ed. Mack, Easton, PA (1990). As used herein, the term "therapeutically effective amount" refers to that amount of HEK4 binding protein which provides a therapeutic effect for a given condition and administration regimen. Said therapeutically effective amount may vary from 0.01 µg/kg body weight to 10 mg/kg body weight and may be determined by one skilled in the art.

HEK4 binding protein may be administered by injection, either subcutaneous, intravenous or intramuscular, or by oral or nasal administration. The route of administration to be chosen will depend upon several variables, including the nature and severity of the condition being treated and the pharmacokinetic properties of the HEK4 binding protein preparation. HEK4 binding protein may be formulated for delivery in a particular fashion, e.g., it may be modified with water soluble polymers, such as polytheylene glycol to improve properties for nasal delivery or to improve serum half-life after injection; or it may be incorporated into particulate preparations of polymeric compounds (e.g., liposomes)

for controlled delivery over an extended period of time.

The expression of HEK4 receptor and HEK4 BP in various tissues is reported in Examples 6A and 6B, respectively. HEK4 receptor mRNA was most abundant in human placenta and was also detected in heart, brain, lung, liver, muscle, kidney tissues. HEK4 BP mRNA was most abundant in human adult brain, kidney and placenta, and was detected at lower levels in heart, lung, liver, spleen, prostate, testis, ovary, small intestine, muscle, pancreas and colon. These patterns of expression suggest that activation of HEK4 receptor by HEK4 BP modulates the growth and/or differentiation of a variety of target cells, particularly those in the brain, heart, lung, liver, muscle and pancreas where expression of both receptor and ligand are detected. In addition, Wicks et al., supra has reported HEK4 receptor mRNA in pre-B and T cell lines, suggesting a role for HEK4 BP in hematopoiesis.

As described in Example 7, HEK4 BP also activates ECK receptor in a cell-cell autophosphorylation assay. Eck receptor mRNA is most abundant in adult rat lung, small intestine, kidney, ovary and skin with lower levels detected in brain, spleen and submaxillary gland (Lindberg and Hunter, supra). Recently, it has been shown that Eck is expressed in the nervous system of the early mouse embryo (Becker et al. Mech. Dev. 47, 3-17 (1994); Ganju et al. Oncogene 9, 1613-1624 (1994)). These observations suggest that activation of ECK receptor by HEK4 BP may modulate the growth and/or differentiation of cells expressing ECK, such as those in the lung, intestine, kidney, skin and nervous system.

Therefore, HEK4 BP is useful in modulating (i.e., increasing or decreasing) the extent of growth and/or differentiation of target cells in various tissues. The target cells will have at least one receptor which is activated by HEK4 BP wherein the receptor is preferably a member of the EPH sub-family of receptor PTKs. Potential therapeutic uses for HEK4 BP are described below.

One aspect of the invention is the use of HEK4 BP to modulate cell growth and differentiation in the nervous system. HEK4 BP may be used to maintain or restore cellular function in the nervous system of a mammal which has been decreased or eliminated by disease or injury or is at risk of being decreased or eliminated by disease or injury. Target cells include neurons and glial cells. Conditions that may be treated by HEK4 BP include central nervous system disorders such as Alzheimer's disease, Parkinson's disease, multiple sclerosis, stroke and Huntington's disease and peripheral nervous system disorders such as amyotrophic lateral sclerosis (ALS) and peripheral neuropathies. Physical injuries to the spinal cord and to peripheral neurons may also be treated with HEK4 BP.

Another aspect of the invention is the modulation by HEK4 BP of growth and differentiation of digestive tract (including large and small intestine), liver, lung, pancreas, muscle and hematopoietic tissues. This activity of HEK4 BP may be particularly useful in regeneration of tissue in these and other sources which has been damaged or depleted by disease or injury.

It has been observed that the sub-family of EPH-like receptors and their corresponding ligands are highly expressed in some carcinoma cell lines (see for example, PCT Application No. WO94/11020 for expression

of ECK receptor and ECK binding protein in human carcinoma cell lines). Thus another aspect of the invention is the treatment of cancers using HEK4 BP antagonists to block cell proliferation. Such cancers are likely to be associated with organs which express HEK4 receptors and/or Eck receptors. HEK4 BP antagonists may be any compound which blocks the biological activity of HEK4 BP and may include, but are not limited to, the following: antibodies which bind to either HEK4 BP or to an EPH sub-family receptor which is activated by HEK4 BP such that a receptor/ligand interaction is prevented; HEK4 BP which binds to, but does not activate and EPH-like receptor; and soluble EPH-like receptors which bind to HEK4 BP. It is envisioned that small molecule mimetics of the above described antagonists are also encompassed by the invention.

In addition to in vivo applications, HEK4 BP may also be used ex vivo to amplify cell populations prior to transplantation. It is envisioned that HEK4 BP may promote growth in culture of cells from the digestive tract, liver, lung, bone marrow, kidney, or central and peripheral nervous systems (and glial cells) neurons such that the amplified population can be introduced back into a patient in need of such therapy. Such so-called "cell therapy" is useful in replenishing cells after damage or depletion and may be appropriate under conditions where systemic administration of HEK4 BP is not preferred.

HEK4 BP may be used alone or in combination with other therapeutic agents for the treatment of cancer, neurological disorders, disorders of the digestive tract, liver, or lung, and for the ex vivo expansion of cell populations. HEK4 BP may be used in conjunction with other chemotherapeutic drugs or with

radiation therapy for the treatment of cancer, or with other neurotrophic factors such as brain derived neutrophic factor (BDNF), ciliary neurotrophic factor (CNTF), neurotrophin-3 (NT-3), nerve growth factor (NGF), or glial derived neurotrophic factor (GDNF) for neurological disorders; or with tissue growth factors such as platelet derived growth factor (PDGF), fibroblast growth factor (FGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF) or keratinocyte growth factor (KGF) for restoration of damaged or depleted tissues.

Isolated nucleic acids of the present invention are useful reagents for the detection and quantitation of DNA and/or RNA coding for HEK4 BP by standard hybridization procedures such as those described in Sambrook et al. Molecular Cloning, A Laboratory Manual, 2d ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1989). These reagents allow one to determine the potential of various cell types to express HEK4 BP and related polypeptides and are also useful for detecting abnormalities in genes encoding HEK4 BP or in sequences controlling the expression of HEK4 BP. Nucleic acids of the invention are also useful for controlling expression levels of HEK4 BP. So-called "anti-sense" nucleic acids hybridize to DNA and/or RNA strands encoding HEK4 BP in a manner that blocks transcription or translation of HEK4 BP nucleic acid sequences. Introduction of HEK4 BP anti-sense nucleic acids into cells overexpressing HEK4 BP is appropriate when such overexpression leads to undesirable physiological effects, such as excessive cell proliferation.

Antibodies which specifically bind HEK4 BP are useful reagents for the detection and quantitation

of HEK4 BP in biological samples using immunoassays (Western blots, RIAs, ELISAs) that are conventional to the art. The presence of HEK4 BP may be indicative of cell proliferation or the potential for cell proliferation, and elevated levels may signal abnormal cell growth typically associated with cancer. In addition, antibodies of the invention may also be useful therapeutic reagents that act as agonists or antagonists of HEK4 BP activity. Antibodies may bind to HEK4 BP in a manner that directly or indirectly blocks HEK4 BP binding an EPH receptor (either HEK4 or ECK). Alternatively, antibodies may bind to HEK4 BP in a manner which promote receptor binding and activation by, for example, "clustering" HEK4 BP into a dimeric or higher multimeric forms to allow more efficient binding and activation of receptor. Antibodies can be monoclonal, polyclonal, or recombinant.

The following examples are offered to more fully illustrate the invention, but are not construed as limiting the scope thereof.

EXAMPLE 1

Production of HEK4 receptor extracellular doman (HEK4-X)

A cDNA clone coding for the HEK4 receptor protein tyrosine kinase was isolated from a human fetal brain cDNA library (Stratagene, La Jolla, CA) as described in co-pending and commonly owned U.S. Serial No. 08/229,509. The sequence of this clone was identical to that in Figure 1 of Wicks et al., supra with the following exc ptions. Wicks reported the

sequence TTA at nucleotides 1618-1620 whereas the HEK4 receptor clone isolated as described here had the sequence TTC at these positions. However, Wicks et al.'s predicted protein sequence specifies a phenylalanine residue in this position, which is inconsistent with an "A" at nucleotide 1620 (TTA codes for leucine while TTC codes for phenylalanine). Also, nucleotides 1529 through 1531 of the Wicks et al. sequence are absent from the sequence obtained here. This change does not affect the translational reading frame, but does eliminate the predicted glutamine residue at position 478 of the Wicks sequence. The effect of these differences on the biological activity of the receptor or the ability to bind ligand is unknown.

The HEK4 receptor cDNA clone was used as a template in a polymerase chain reaction (PCR) designed to amplify a DNA fragment coding for the ligand binding domain of the HEK4 receptor. The primers used were:

433-26) 5' GGATCTAGAGCACCAGCAACATGGATTGT 3'
(SEQ ID NO: 3)

409-10) 5' TCGGTCTAGATCATTATTGGCTACTTTCACCAGAGAT 3'
(SEQ ID NO: 4)

These primers produce a fragment 1656 nucleotides in length that codes for a protein of 540 amino acids. The predicted protein consists of the entire extracellular domain of the HEK4 receptor from the amino terminus up to but not including the transmembrane region. The 1656 nucleotide fragment was digested with the restriction endonuclease XbaI and ligated into the expression vector pDSRα which had been digested with the same enzyme. The resulting

expression plasmid was introduced into CHO cells by calcium phosphate mediated transfection (Cellphect, Pharmacia, Piscataway, NJ). Individual colonies were selected based upon the expression of the dihydrofolate reductase (DHFR) gene which resides on the expression plasmid. Expression of the HEK4 gene was monitored by RNA solution hybridization (Hunt et al. Exp. Hematol. 19, 779-784 (1991)) and/or by Western blotting with antibodies directed against amino acids 22-148 of the HEK4 extracellular domain.

HEK4 expression was enhanced by growth of the selected clones in 100 nM methotrexate. One of the pDSRα/HEK4-X clones was chosen for large scale production. Twenty-four roller bottles were seeded at a density of approximately $2x10^7$ cells/bottle in 200 ml each of Dulbecco's Minimal Essential Media (DMEM) supplemented with non-essential amino acids (1X NEAA, Gibco), 100 nM methotrexate, 1X penicillin/streptomycin/glutamine (1X PSG, Gibco) and 10% fetal bovine serum. Cells reached confluence in 3-4 days at which time the media was changed to DMEM/NEAA/PSG lacking serum. Cell-conditioned media was harvested after seven days, concentrated, and diafiltered against 10 mM Tris-HCl, pH 8.5. The concentrated media was loaded onto a Q-sepharose FF (Pharmacia) ion exchange column and bound material was eluted with a linear gradient of 0 to 0.5 M NaCl in 10 mM Tris-HCl, pH 8.5. Fractions were analyzed by SDS-PAGE and western blotting using a rabbit polyclonal antibody directed against residues 22-148 of the HEK4 external domain. Fractions containing HEK4-X protein were pooled, concentrated and loaded onto an S-200 (Sephaoryl S-200, Pharmacia) column. Fractions from this column were analyzed as before and those containing HEK4-X were pooled.

EXAMPLE 2

A. Purification of HEK4-X Binding Activity

We have previously described the use of the BIAcore™ instrument (Pharmacia Biosensor, Piscataway, NJ) for the detection of receptor binding activity in concentrated cell-conditioned media (Bartley et al., supra). We used a similar strategy to screen for HEK4 receptor binding activity as described below.

The surface of a BIAcore sensor chip was activated by injection of 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-HCl and 0.05 M N-hydroxysuccinimide at a flow rate of 5 ul/min. Purified HEK4-X at a concentration of 250 ug/ml was applied to the activated surface in two 50 ul injections at the same flow rate. Unreacted binding sites were blocked by injection of 1M ethanolamine, pH 8.5. The surface was washed overnight in 10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween 20, pH 7.4 until the baseline was stable. Typically, immobilization resulted in 6000-8000 resonance units (RU) of HEK4-X bound to the sensor chip.

Conditioned media samples were collected from 108 cell lines grown either without fetal bovine serum (FBS) or in the presence of 0.5% FBS. Conditioned media produced under serum-free conditions was adjusted to 0.5% FBS before further processing. The media was filtered, concentrated 25-fold, and stored in aliquots at -80°C. 30ul samples of each medium were injected onto the HEK4-X surface at a flow rate of 5 µl/min and the binding response measured 20 seconds after the conclusion of each injection. Between samples, the surface was regenerated by 10-15 µl injections of 25 mM 3-(cyclohexylamino)-1-propanesulfonic acid, pH 10.4.

Concentrated conditioned media samples displaying binding of 200 resonance units or more are listed in Table 1.

Table 1

Cell Line	Description	Binding (resonance units)
HCT116	human colon carcinoma	911
M-14	human melanoma	337
LS174T	human colon adenocarcinoma	316
A498	human kidney carcinoma	274
A172	human glioblastoma	269
PK(15)1	porcine kidney	234
JEG-1	human choriocarcinoma	220
Y-79	human retinoblastoma	216
HT 1080	human fibrosarcoma	200

The five conditioned media displaying the most binding were selected for further investigation. When soluble dextran was added to samples before injection to reduce non-specific binding, the signals from HCT116 and LS174T conditioned media were greatly reduced. A172 cells proved difficult to grow and were unsuitable for the large scale production of conditioned media. Based on these experiments and on pilot scale receptor affinity chromatography, the A498 cell line (ATCC No. HTB 44) was chosen as the best source of conditioned media for purification of a HEK4 binding protein.

A HEK4 receptor affinity column was prepared by immobilization of HEK4-X on CNBr-activated Sepharose 4B (Pharmacia). Purified HEK4-X was dialyzed against 0.1 M $NaHCO_3$, 0.5 M NaCl, pH 8.3 and

brought to a final concentration of 2 mg/mL. Immobilization of HEK4-X was done at a ligand density of 1 mg/mL according to the method of Kenny et al. (New Protein Techniques, J.M. Walker, ed. The Humana Press, Clifton, NJ 1988). Forty liters of A498 conditioned media produced in 0.5% serum-containing media was concentrated 40-fold, diafiltered against PBS and 0.02% NaN_3, and loaded onto the HEK4-CNBr sepharose column. The column was washed with PBS and bound material was eluted with 50 mM sodium acetate, 0.5 M sodium chloride, pH 4.0. Fractions were collected in 1 mM CHAPS and loaded directly on polyacrylamide gels. Gels were either stained with silver for analysis or blotted onto PVDF membranes (Problot, Applied Biosystems, Foster City, CA) in preparation for N-terminal amino acid sequencing (Fausset & Lu, 1991).

The pH 4.0 elution fractions from the HEK4-CNBr Sepharose column contained three major protein species with molecular weights of 21, 25 and 27 kD which were not apparent in the load or wash fractions. The fractions containing these 3 proteins were pooled, concentrated in the presence of CHAPS and applied to a Vydac C4 reverse phase HPLC column (4.6 x 150 mm). The column was eluted with a gradient of acetonitrile (26-35%) in 0.1% trifluoroacetic acid. Fractions were collected, volume reduced under vacuum, and analyzed for HEK4 binding protein. The three major peaks detected by absorbance at 214 nm were pooled and analyzed by SDS-PAGE (see Figure 2). Further purification of the three isoforms of HEK4 BP was achieved by reapplying the proteins to the same C4 column.

B. Sequencing of peptides

A sample from the initial purification of A498 cell-conditioned media was submitted for protein sequencing. The sample was analyzed on SDS-PAGE and blotted onto PVDF membrane. The gel band identified as the HEK4 binding protein was excised and analyzed for five cycles on an Applied Biosystems 477A protein sequencer. This yielded no sequence, indicating that the protein was N-terminally blocked. The sample was then treated with cyanogen bromide and reapplied to the sequencer. A tentative sequence was obtained from the cleaved sample by assuming the highest yield at each cycle to belong to the same peptide. Even given this assumption, recoveries were so small as to render the sequence unreliable after cycle 10. The sequence obtained in this manner is shown as peptide #1 in Table 2.

Table 2

Peptide No.	Amino acid sequence
1	Val-Asn-Phe-Asp-Gly-Tyr-Ser-Ala-Arg-Asp (SEQ ID NO: 5)
2	Val-Phe-Asp-Val-Asn-Phe-Lys-Val-Glu-X-Ser-Leu-Glu-Pro-Ala-Asp (SEQ ID NO: 6)
3	Ala-Val-Ala-Asp-Arg-Tyr-Ala-Val-Tyr-Trp-Asn-Ser-Ser-Asn-Pro-Arg-Phe-Gln-Arg-Gly-Asp-Tyr-His-Ile-Ile-Val-X-Ile-Asn-X-Tyr (SEQ ID NO: 7)

Subsequent analysis of samples cleaved by cyanogen bromide, then separated by SDS-PAGE indicated that position 9 of peptide #1 was a cysteine residue and position 6 of peptide #2 was aspartic acid. Position 25 of peptide #3 was subsequently found by DNA sequencing to be aspartic acid. The sequence data shown in peptides #2 and #3 in Table 2 was obtained by analysis of tryptic digests of the protein followed by separation of the resulting peptides on a microbore C4 column. These experiments were done with larger amounts of starting material and therefore yielded more reliable sequence and allowed 20-30 cycle sequencing runs. Comparison of the peptide sequences in Table 2 with B61 suggests that they represent fragments of a related protein. We therefore conclude that the HEK4 binding protein is another ligand for the human EPH-like kinase sub-family.

EXAMPLE 3

A. Cloning and Sequencing of cDNA Encoding HEK4 Binding Protein

The amino acid sequences obtained from HEK4 BP peptides as shown in Table 2 were used to design oligonucleotide primers. Primers 702-3 and 633-11 were used in a PCR reaction with random primed A498 cDNA as a template.

702-3) 5' GAYMGNTAYGCNGTNTAYTGG 3' (SEQ ID NO:8)

633-11) 5' RTANCCRTCRAARTTNACCAT 3' (SEQ ID NO:9)

The 175 base pair fragment amplified by these primers was sequenced and found to be closely related to B61. This fragment was then radiolabeled with ^{32}P by random

priming and used as a probe to screen a cDNA library for clones containing the full length HEK4 BP cDNA. An oligo-dT primed human placental cDNA library purchased from Stratagene (La Jolla, CA) was plated at a density of 30,000 plaques/150 mm plate. Replicas of the plaques arrayed on the plates were made on GeneScreen™ hybridization transfer membranes (New England Nuclear, Boston, MA) as directed by the manufacturer. Two replica filters were made for each plate. Filters were pre-hybridized in 6X SSC, 1X Denhardts buffer, 50 ug/ml salmon testis DNA, 1% SDS at 65°C for 4 hours followed by hybridization with the ^{32}P-labeled probe for 12 hours under the same conditions. Following hybridization, filters were washed two times, 1 hour each, in 0.2X SSC, 0.5% SDS at 65°C and exposed to Kodak XAR film overnight with an intensifying screen. Comparison of the two filters made from each plate showed that five plaques were positive on both replicas. The phage around each positive plaque were removed, resuspended in buffer, and replated at a lower density to produce well-separated plaques for secondary screening. Individual plaques which were positive upon rescreening (using the same method as the primary screen) were picked. The inserts from these phage were transferred into the pBluescript plasmid by in vivo excision as described by the manufacturer (Stratagene). Three of the five inserts were identical and contained the entire coding region of HEK4 BP while the other two represented overlapping clones. A consensus sequence was assembled using data from the three inserts containing the entire coding region and is shown in Figure 3.

The HEK4 BP cDNA sequence predicts a protein of between 213 and 228 amino acids, depending on which of three possible initiator codons is utilized. Based upon rules for translation of

vertebrate mRNAs (Kozak Cell 44, 283-292 (1986)), the third in-frame ATG is an unlikely initiator, while the first ATG, being the farthest upstream is the most likely to be the principal initiation codon. As for B61, HEK4 BP has hydrophobic amino acids on both the amino and carboxy termini. These probably function as a secretion signal sequence and a membrane anchor, respectively. Like B61, HEK4 BP apparently has both soluble and membrane bound forms. Although we were not able to obtain N-terminal protein sequence data, we would predict cleavage of the signal peptide to yield a mature protein with serine at position 1 (Figure 3). Based on peptide mapping and mass spectrometric analysis, proline-179 (Figure 3) appears to be the C-terminal amino acid in the major soluble form found in A498 cell-conditioned media. An alternate form with alanine-177 (Figure 3) at the C-terminus was also detected.

B. Expression of recombinant HEK4 BP

The HEK4 BP cDNA clone shown in Figure 3 was inserted into the plasmid vector pDSRα for expression mammalian cells. The recombinant plasmid was transfected into Chinese hamster ovary (CHO) cells by calcium phosphate precipitation and cells containing the plasmid were selected by growth in DMEM (high glucose, GIBCO, Bethesda, MD), 1X penicillin/streptomycin/glutamine (PSG), 1X non-essential amino acids (NEAA) containing 10% fetal bovine serum (FBS), but lacking HT supplement (HT supplement: 10 mM sodium hypoxanthine, 1.6 mM thymidine). The expression of HEK4 BP in several clones was assessed by the level of HEK4-receptor binding activity in each clone's cell-conditioned media as determined by BIAcore (Pharmacia Biosensor, Piscataway, NJ). This correlated well with the level

of HEK4 BP mRNA in the clones as determined by Northern blot hybridization. One clone, CHO/HL6, was a significantly better producer of recombinant HEK4 BP than the others and was chosen for further work. Expression of HEK4 BP by CHO/HL6 was enhanced 2 to 4-fold by treatment with increasing amounts of methotrexate up to 100 nM over a period of several weeks. Following amplification, cells expressing HEK4 BP were expanded and transferred to roller bottles for production of conditioned media to be used as a source for purification of the recombinant protein. A total of 100 roller bottles were seeded with CHO/HL6 cells at 10^7 cells per bottle. Cells were grown until confluent (approximately 4 days) in DMEM (high glucose, GIBCO, Bethesda, MD) , 1X PSG, 1X NEAA, and 10% FBS. Following the growth phase, the media was removed replaced with the same media but with 0.5 % rather than 10% FBS. After 3 days, the media was collected, filtered to remove any cell debris, and stored frozen at -80°C.

EXAMPLE 4

Purification of recombinant HEK4 BP

Approximately 20-25 liters of conditioned medium from CHO/HL6 cells were thawed at room temperature and filtered. The medium was concentrated and diafiltered against 10mM Tris-HCl, pH 8.5 (4°C) using a 10,000 molecular weight cut off membrane. The diafiltrate was applied to a column of Q-Sepharose, High Performance and subsequently eluted with a linear gradient of NaCl (0-0.3 M) in 10 mM Tris-HCl, pH 8.5. Fractions were analyzed for the presence of HEK4 BP by immunoblotting using an antibody generated against

unfolded HEK4 BP produced in E. coli, or by binding to HEK4 receptor immobilized on a BIAcore sensor chip. Fractions containing HEK4 BP were pooled, concentrated and applied to a gel filtration column. (Superdex 75, 5 x 85 cm, PBS, 3 mL/min). Fractions containing HEK4 BP were further purified by C4 reverse phase HPLC (Vydac 214TP 4.6 x 250 mm, 2.9 mL/min) using an acetonitrile gradient (22-44%) in 0.1% trifluoroacetic acid. The column profile and SDS-PAGE analysis of peak fractions are shown in Figure 4. Fractions were evaporated under vacuum and formulated in 0.25 M Tris-HCl, 2 mM CHAPS, pH 7.5.

EXAMPLE 5

A. Production of Antibodies to HEK4 Receptor

Antibodies directed against the HEK4 receptor extracellular domain were produced using standard methods (Harlow and Lane, Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY (1988)). cDNA encoding amino acids 22-148 of the HEK4 receptor was inserted into the pATH vector (Yansura, 1990) in the same translational reading frame as the TrpE gene. The resulting plasmid was introduced into an E. coli host strain resulting in expression of the HEK4/TrpE fusion protein. Bacterial cell lysates were fractionated by preparative SDS-PAGE and the band containing the HEK4/TrpE fusion was excised. The crushed gel slice was used to immunize rabbits according to standard protocols (Harlow and Lane, supra). The antisera generated by this method recognized both the HEK4/TrpE antigen and recombinant HEK4-X produced in CHO cells.

Antibodies to the C-terminal 12 amino acids of the HEK4 receptor (sequence is cys-leu-glu-thr-gln-

ser-lys-asn-gly-pro-val-pro-val) were produced by the same method (Harlow and Lane, supra) using a synthetic peptide chemically linked to keyhole limpet hemocyanin (KLH) as the antigen. The antiserum was purified by passage over a column upon which peptide antigen had been immobilized using a SulfoLink kit (Pierce, Rockford, IL). These antibodies were able to specifically recognize HEK4 receptor by Western blots.

B. Production of Antibodies to HEK4 BP

HEK4 BP cDNA as shown in Figure 3 was used as a template for PCR with primers 819-31 and 819-28 to produce a polypeptide fragment coding for amino acids 1-179 of HEK4 BP (Figure 3).

819-31) 5' GGAGGACATATGAGCCAGGACCCGGGCTCCAAG 3'
(SEQ ID NO:10)

819-28) 5' GAAGAAGGATCCCTATGGCTCGGCTGACTCATGTAC 3'
(SEQ ID NO:11)

The PCR fragment was cloned into the expression vector pCFM1656 using the NdeI and BamHI sites included in the primers. The resulting recombinant plasmid was transformed into E. coli FM5 (ATCC No. 53911) and the truncated HEK4 BP was expressed as insoluble inclusion bodies. The inclusion bodies were solubilized and the HEK4 BP fragment purified by SDS-polyacrylamide gel electrophoresis was used as an antigen in rabbits. The antisera was generated and characterized as described (Harlow and Lane, supra) and recognized HEK4 BP expressed in CHO cells by Western blot analysis.

EXAMPLE 6

A. Expression pattern of the HEK4 receptor

The expression of HEK4 receptor mRNA in rat and human tissues has been previously reported in co-pending and commonly owned U.S. Serial No. 08/229,509, relevant portions of which have been incorporated herein by reference. The results of these studies are summarized in Table 3.

TABLE 3

Tissue Distribution of HEK4 Receptor

Tissue:	Human	Rat
Brain	++	+
Heart	+	bd
Kidney	+	bd
Liver	+	bd
Lung	+	+
Muscle	+	bd
Ovary	nt	bd
Pancreas	+	nt
Placenta	+++	nt
Stomach	nt	bd
Testis	nt	+
Thymus	nt	bd

bd = below detection
nt = not tested

In the human tissues studied, HEK4 receptor mRNA is most abundantly expressed in placenta, with lower levels in heart, brain, lung, and liver. Previous studies on HEK4 receptor mRNA in cell lines found

expression in one pre-B cell line and two T-cell lines (Wicks et al. 1992).

B. Expression pattern of HEK4 BP

The expression of HEK4 BP mRNA in human tissues was examined by Northern blot analysis. A Northern blot containing 2 μg of polyA$^+$ from each of the tissues indicated was purchased from Clontech (Palo Alto, CA) and hybridized with a ^{32}P-labeled HEK4 BP probe. As shown in Figure 5, HEK4 BP mRNA is expressed at high levels in human adult brain, kidney, and placenta. Readily detectable levels can also be found in heart, lung, liver, spleen, prostate, testis, ovary, small intestine, and colon. The presence of HEK4 BP mRNA in many different tissues is consistent with the idea that this factor is important for the differentiation, development, and/or maintenance of a variety of cell types.

EXAMPLE 7

A. HEK4 BP activation of EPH subfamily receptors by cell-cell autophosphorylation

The hallmark of receptor activation for all known receptor protein-tyrosine kinases is autophosphorylation (van der Geer et al., supra). To determine whether HEK4 BP can activate HEK4 receptor, a cell-cell autophosphorylation assay was performed. Recipient cells were CHO cells transfected with HEK4 receptor cDNA which had been serum-starved by incubation in media with 0.5% serum for 16 hours. The donor cells were CHO cells transfected with HEK4 BP cDNA (see Example 3B) or CHO cells that had been transfected with vector alone. Donor cells were scraped from the surface of their growth vessel in

phosphate-buffered saline and added to recipient cells for 30 minutes at 37°C. After washing, the recipient cells were lysed in modified RIPA buffer (10 mM sodium phosphate, pH 7.4, 150 mM sodium chloride, 0.1% sodium dodecyl sulfate, 1% NP-40, 1% deoxycholate, 10 mg/ml aprotinin, 5mM EDTA, 200 mM sodium orthovanadate). Receptors were immunoprecipitated from the cell lysate and prepared for SDS polyacrylamide gel electrophoresis as previously described (Bartley et al., supra). After electrophoresis and electroblotting to membranes, the immunoprecipitates were probed with antiphosphotyrosine antibodies (4G10, UBI, Lake Placid, NY). Immune complexes were detected by horseradish peroxidase conjugated secondary reagents using chemiluminescence as described by the manufacturer (ECL, Amersham). As shown in Figure 6, cells expressing HEK4 binding protein were able to stimulate tyrosine phosphorylation on both the HEK and ECK receptors. Control cells did not stimulate the phosphorylation of either receptor. The results demonstrate that HEK4 BP can activate both the HEK4 and the ECK receptors.

B. Activation of HEK4 Receptor by Soluble HEK4 BP

To determine whether soluble recombinant HEK4 BP could activate the HEK4 receptor, CHO cells transfected with HEK4 receptor cDNA were treated with conditioned media from CHO cells expressing HEK4 BP (see Example 3B) or with purified recombinant HEK4 BP (see Example 4). The cells were serum-starved by incubation in media with 0.5% serum for 16 hours prior to the treatments. Treatments were for 30 minutes at 37°C, after which the cells were lysed in modified NP40 buffer (50 mM Tris, pH 8.0, 150 mM sodium chloride, 1% NP40, 10 mg/ml aprotinin, 5mM EDTA, 200 mM sodium orthovanadate), HEK4 receptor was

immunoprecipitated, and prepared for SDS polyacrylamide gel electrophoresis as previously described (Bartley et al., supra). After electrophoresis and electroblotting to membranes, the immunoprecipitates were probed with antiphosphotyrosine antibodies (4G10, UBI, Lake Placid, NY). Immune complexes were detected by horseradish peroxidase conjugated secondary reagents using chemiluminescence as described by the manufacturer (ECL, Amersham). As shown in Figure 7, soluble recombinant HEK4 BP in conditioned media and after purification activated HEK4 receptor. This activation was enhanced by pretreatment of conditioned media or purified HEK4 BP with the antibodies of Example 5B which had been affinity purified on a HEK4 BP column. Antibodies (~50 µg/ml) were incubated with conditioned medium or purified HEK4 BP at 4° for 1 hr. prior to treatment of CHO cells expressing HEK4 receptor.

EXAMPLE 8

Affinity of HEK4 BP for EPH-like Receptors

A competition assay was used to measure differences in HEK4 BP binding to different EPH-like receptors. Purified HEK4 BP was incubated with various concentrations of either HEK4, HEK8 or ECK soluble receptors and binding of the mixture to immobilized HEK4 receptor was analyzed by BIAcore. The concentration of soluble receptor that inhibited HEK4 BP binding by 50% is termed IC50. IC50 values allow a comparison of the relative affinity of HEK4 BP for related receptors.

IC50 values were determined as follows. Analysis of HEK4 BP binding to immobilized HEK4 receptor showed a linear response in the range 60 to 500 ng/ml. Various amounts of the purified extracellular domains of HEK4, ECK, or HEK8 were incubated with 0.250 µg/ml HEK4 BP prepared as described in Example 4 in solutions containing 100 µg/ml BSA, 10mM HEPES, 0.15M NaCl, 3.4 mM EDTA, and 1 mg/ml soluble dextran, pH 7.4. These solutions were incubated for at least 30 minutes at 3°C prior to injection. Protein concentrations of receptor stocks were confirmed by BCA protein assay. Duplicates of each sample were run in parallel with standard curves on two different days. All surfaces were regenerated to within 10 RU of baseline with 25mM CAPS and 1M NaCl pH 10.4. The mean binding response was plotted versus soluble receptor concentration (Figure 8) providing IC50 values of 0.55 ug/ml for HEK4, 5.0 µg/ml for ECK, and 10.5 µg/ml for HEK8. Thus, HEK4 BP preferentially binds HEK4 receptor compared to two other EPH family members, ECK and HEK8.

* * *

While the invention has been described in what is considered to be its preferred embodiments, it is not to be limited to the disclosed embodiments, but on the contrary, is intended to cover various modifications and equivalents included within the spirit and scope of the appended claims, which scope is to be accorded the broadest interpretation so as to encompass all such modifications and equivalents.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Amgen Inc.

(ii) TITLE OF INVENTION: Ligands for EPH-Like Receptor

(iii) NUMBER OF SEQUENCES: 11

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 91320-1789

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Winter, Robert B.
(C) REFERENCE/DOCKET NUMBER: A-325

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1728 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 175..858

(ix) FEATURE:
(A) NAME/KEY: mat_peptide
(B) LOCATION: 232..858

(ix) FEATURE:
(A) NAME/KEY: sig_peptide
(B) LOCATION: 175..231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GAATTCCCCC AGCTTGGTGG GCGCCTCTTT CCTTTCTCGC CCCCTTTCAT TTTTATTTAT      60

TCATATTTAT TTGGCGCCCG CTCTCTCTCT GTCCCTTTGC CTGCCTCCCT CCCTCCGGAT     120

CCCCGCTCTC TCCCCGGAGT GGCGCGTCGG GGGCTCCGCC GCTGGCCAGG CGTG ATG       177
                                                            Met
                                                            -19

TTG CAC GTG GAG ATG TTG ACG CTG GTG TTT CTG GTG CTC TGG ATG TGT       225
Leu His Val Glu Met Leu Thr Leu Val Phe Leu Val Leu Trp Met Cys
          -15                 -10                 -5

GTG TTC AGC CAG GAC CCG GGC TCC AAG GCC GTC GCC GAC CGC TAC GCT       273
Val Phe Ser Gln Asp Pro Gly Ser Lys Ala Val Ala Asp Arg Tyr Ala
     1               5                  10

GTC TAC TGG AAC AGC AGC AAC CCC AGA TTC CAG AGG GGT GAC TAC CAT       321
Val Tyr Trp Asn Ser Ser Asn Pro Arg Phe Gln Arg Gly Asp Tyr His
 15                  20                  25                  30

ATT GAT GTC TGT ATC AAT GAC TAC CTG GAT GTT TTC TGC CCT CAC TAT       369
Ile Asp Val Cys Ile Asn Asp Tyr Leu Asp Val Phe Cys Pro His Tyr
                35                  40                  45

GAG GAC TCC GTC CCA GAA GAT AAG ACT GAG CGC TAT GTC CTC TAC ATG       417
Glu Asp Ser Val Pro Glu Asp Lys Thr Glu Arg Tyr Val Leu Tyr Met
            50                  55                  60

GTG AAC TTT GAT GGC TAC AGT GCC TGC GAC CAC ACT TCC AAA GGG TTC       465
Val Asn Phe Asp Gly Tyr Ser Ala Cys Asp His Thr Ser Lys Gly Phe
        65                  70                  75

AAG AGA TGG GAA TGT AAC CGG CCT CAC TCT CCA AAT GGA CCG CTG AAG       513
Lys Arg Trp Glu Cys Asn Arg Pro His Ser Pro Asn Gly Pro Leu Lys
    80                  85                  90

TTC TCT GAA AAA TTC CAG CTC TTC ACT CCC TTT TCT CTA GGA TTT GAA       561
Phe Ser Glu Lys Phe Gln Leu Phe Thr Pro Phe Ser Leu Gly Phe Glu
 95                 100                 105                 110

TTC AGG CCA GGC CGA GAA TAT TTC TAC ATC TCC TCT GCA ATC CCA GAT       609
Phe Arg Pro Gly Arg Glu Tyr Phe Tyr Ile Ser Ser Ala Ile Pro Asp
                115                 120                 125

AAT GGA AGA AGG TCC TGT CTA AAG CTC AAA GTC TTT GTG AGA CCA ACA       657
Asn Gly Arg Arg Ser Cys Leu Lys Leu Lys Val Phe Val Arg Pro Thr
            130                 135                 140

AAT AGC TGT ATG AAA ACT ATA GGT GTT CAT GAT CGT GTT TTC GAT GTT       705
Asn Ser Cys Met Lys Thr Ile Gly Val His Asp Arg Val Phe Asp Val
        145                 150                 155
```

```
AAC GAC AAA GTA GAA AAT TCA TTA GAA CCA GCA GAT GAC ACC GTA CAT        753
Asn Asp Lys Val Glu Asn Ser Leu Glu Pro Ala Asp Asp Thr Val His
    160                 165                 170

GAG TCA GCC GAG CCA TCC CGC GGC GAG AAC GCG GCA CAA ACA CCA AGG        801
Glu Ser Ala Glu Pro Ser Arg Gly Glu Asn Ala Ala Gln Thr Pro Arg
175                 180                 185                 190

ATA CCC AGC CGC CTT TTG GCA ATC CTA CTG TTC CTC CTG GCG ATG CTT        849
Ile Pro Ser Arg Leu Leu Ala Ile Leu Leu Phe Leu Leu Ala Met Leu
                195                 200                 205

TTG ACA TTA TAGCACAGTC TCCTCCCATC ACTTGTCACA GAAAACATCA                 898
Leu Thr Leu

GGGTCTTGGA ACACCAGAGA TCCACCTAAC TGCTCATCCT AAGAAGGGAC TTGTTATTGG      958

GTTTTGGCAG ATGTCAGATT TTTGTTTTCT TTCTTTCAGC CTGAATTCTA AGCAACAACT     1018

TCAGGTTGGG GGCCTAAACT TGTTCCTGCC TCCCTCACCC CACCCCGCCC CACCCCCAGC     1078

CCTGGCCCTT GGCTTCTCTC ACCCCTCCCA AATTAAATGG ACTCCAGATG AAAATGCCAA     1138

ATTGTCATAG TGACACCAGT GGTTCGTCAG CTCCTGTGCA TTCTCCTCTA AGAACTCACC     1198

TCCGTTAGCG CACTGTGTCA GCGGGCTATG GACAAGGAAG AATAGTGGCA GATGCAGCCA     1258

GCGCTGGCTA GGGCTGGGAG GGTTTTGCTC TCCTATGCAA TATTTATGCC TTCTCATTCA     1318

GAACTGTAAG ATGATCGCGC AGGGCATCAT GTCACCATGT CAGGTCCGGA GGGGAGGGCC     1378

TATCCCCCTA TCCCAGGCAT CCCAGACGAG GACTGGCTGA GGCTAGGCGC TCTCATGATC     1438

CACCTGCCCC GGGAGGGCAG CGGGGAAGAC AGAGAAAAGC AAAACGCATT CCTCCTCAGC     1498

TCCACCCACC TGGAGACGAA TGTAGCCAGA GAGGAGGAAG GAGGGAAACT GAAGACACCG     1558

TGGCCCCTCG GCCTTCTCTC TGCTAGAGTT GCCGCTCAGA GGCTTCAGCC TGACTTCCAG     1618

CGGTCCCAAG AACACCTACT AATTCTTCTC CACTCCTTCA TGGCTGGGAC AGTTACTGGT     1678

TCATATGCAA GTAAAGATGA CAATTTACTC AACAAAAAAA AAAGGAATTC                 1728
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 228 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Leu His Val Glu Met Leu Thr Leu Val Phe Leu Val Leu Trp Met
-19                 -15                 -10                 -5
```

```
Cys Val Phe Ser Gln Asp Pro Gly Ser Lys Ala Val Ala Asp Arg Tyr
                1               5                  10

Ala Val Tyr Trp Asn Ser Ser Asn Pro Arg Phe Gln Arg Gly Asp Tyr
     15                  20                  25

His Ile Asp Val Cys Ile Asn Asp Tyr Leu Asp Val Phe Cys Pro His
 30                  35                  40                  45

Tyr Glu Asp Ser Val Pro Glu Asp Lys Thr Glu Arg Tyr Val Leu Tyr
                50                  55                  60

Met Val Asn Phe Asp Gly Tyr Ser Ala Cys Asp His Thr Ser Lys Gly
            65                  70                  75

Phe Lys Arg Trp Glu Cys Asn Arg Pro His Ser Pro Asn Gly Pro Leu
         80                  85                  90

Lys Phe Ser Glu Lys Phe Gln Leu Phe Thr Pro Phe Ser Leu Gly Phe
     95                  100                 105

Glu Phe Arg Pro Gly Arg Glu Tyr Phe Tyr Ile Ser Ser Ala Ile Pro
110                 115                 120                 125

Asp Asn Gly Arg Arg Ser Cys Leu Lys Leu Lys Val Phe Val Arg Pro
                130                 135                 140

Thr Asn Ser Cys Met Lys Thr Ile Gly Val His Asp Arg Val Phe Asp
            145                 150                 155

Val Asn Asp Lys Val Glu Asn Ser Leu Glu Pro Ala Asp Asp Thr Val
        160                 165                 170

His Glu Ser Ala Glu Pro Ser Arg Gly Glu Asn Ala Ala Gln Thr Pro
    175                 180                 185

Arg Ile Pro Ser Arg Leu Leu Ala Ile Leu Leu Phe Leu Leu Ala Met
190                 195                 200                 205

Leu Leu Thr Leu
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGATCTAGAG CACCAGCAAC ATGGATTGT 29

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGGTCTAGA TCATTATTGG CTACTTTCAC CAGAGAT 37

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Val Asn Phe Asp Gly Tyr Ser Ala Arg Asp
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Val Phe Asp Val Asn Phe Lys Val Glu Xaa Ser Leu Glu Pro Ala Asp
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ala Val Ala Asp Arg Tyr Ala Val Tyr Trp Asn Ser Ser Asn Pro Arg
1 5 10 15

Phe Gln Arg Gly Asp Tyr His Ile Ile Val Xaa Ile Asn Xaa Tyr
20 25 30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GAYMGNTAYG CNGTNTAYTG G 21

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

RTANCCRTCR AARTTNACCA T 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GGAGGACATA TGAGCCAGGA CCCGGGCTCC AAG 33

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAGAAGGAT CCCTATGGCT CGGCTGACTC ATGTAC 36

WHAT IS CLAIMED IS:

1. A purified and isolated polypeptide capable of binding at least one EPH-like receptor wherein the receptor is the HEK4 receptor.

2. The polypeptide of Claim 1 wherein the binding results in activation of the EPH-like receptor.

3. The polypeptide of Claim 1 which is human.

4. The polypeptide of Claim 1 which is substantially free of other human proteins.

5. The polypeptide of Claim 1 having in glyocosylated form an apparent molecular weight of about 21-27 kD on nonreducing SDS-PAGE.

6. The polypeptide of Claim 1 which is characterized by being a product of procaryotic or eucaryotic expression of an exogenous DNA sequence.

7. The polypeptide of Claim 6 wherein the exogenous DNA is cDNA, genomic DNA or synthetic DNA.

8. The polypeptide of Claim 1 which has at least about 70% homology to the amino acid sequence as shown in Figure 3 (SEQ ID NO: 1).

9. The polypeptide of Claim 1 which has the amino acid sequence of Figure 3 (SEQ ID NO: 1).

10. The polypeptide of Claim 9 further comprising a methionine residue at position -1.

11. The polypeptide of Claim 1 which is soluble or bound to a cell surface.

12. The polypeptide of Claim 1 which is further capable of binding the ECK receptor.

13. The polypeptide of Claim 1 which is multimeric.

14. The polypeptide of Claim 13 selected from the group consisting of: an immunogloblin chimera, an antibody clustered protein, and covalently and noncovalently attached protein monomers.

15. An isolated nucleic acid encoding a polypeptide capable of binding to at least one EPH-like receptor wherein the nucleic acid is selected from the group consisting of:

a) the nucleic acid as shown in Figure 3 (SEQ ID NO: 1);

b) nucleic acids which hybridize under conditions of 6XSSC and 65°C with the coding region of Figure 3 (SEQ ID NO: 1); and

c) nucleic acids which are degenerate to the nucleic acids of (a) and (b).

16. The nucleic acid of Claim 15 which is cDNA, genomic DNA or synthetic DNA.

17. A polypeptide encoded by the nucleic acids of Claim 15.

18. The nucleic acids of Claim 15 which include one or more codons preferred for expression in Escherichia coli.

19. A biologically functional plasmid or viral vector including the nucleic acid of Claim 15.

20. A procaryotic or eucaryotic host cell stably transformed or transfected with a DNA vector of Claim 19.

21. The host cell of Claim 20 which is a CHO cell.

22. The host cell of Claim 20 which is Escherichia coli.

23. A process for the production of a polypeptide capable of binding at least one EPH-like receptor comprising growing under suitable nutrient conditions procaryotic or eucaryotic host cells transformed or transfected with the nucleic acid of Claim 15 and isolating polypeptide products of the expression of the nucleic acids in the vector.

24. A pharmaceutical composition comprising a therapeutically effective amount of the polypeptide of Claim 1 and a pharmaceutically acceptable adjuvant.

25. The composition of Claim 24 wherein the polypeptide is of human origin.

26. An antibody or fragment thereof specifically binding the polypeptide of Claim 1.

27. The antibody of Claim 26 which is a monoclonal antibody.

28. A method of activating at least one EPH-lik receptor comprising contacting the receptor with the polypeptide of Claim 1.

29. A method of modulating the growth or differentiation of cells comprising contacting the cells with the polypeptide of Claim 1.

30. The method of Claim 29 wherein the cells are kidney, lung, liver, skin, digestive tract, glial, nerve, or hematopoietic cells.

31. A method of maintaining or restoring cellular function in the nervous system of a mammal comprising administering a therapeutically effective amount of the polypeptide of Claim 1.

32. A method of regenerating damaged or depleted tissue in a mammal comprising administering a therapeutically effective amount of the polypeptide of Claim 1.

33. The method of Claim 32 wherein the tissue is from liver, lung, digestive tract or nervous system.

34. A method for detecting the presence of a polypeptide capable of binding at least one EPH-like receptor in a biological sample comprising: contacting the sample with the antibody of Claim 26 under conditions allowing for antibody binding to the polypeptide; and detecting the binding of the antibody.

35. A method for detecting in a biological sample the presence of a nucleic acid encoding a

polypeptide capable of binding at least one EPH-like receptor comprising: contacting the sample with the nucleic acid of Claim 15 under conditions allowing for hybridization; and detecting the hybridization of the nucleic acid.

36. An anti-sense nucleotide sequence which hybridizes to the nucleic acid of Claim 15 in a manner that blocks transcription or translation of the nucleic acid.

FIGURE 1

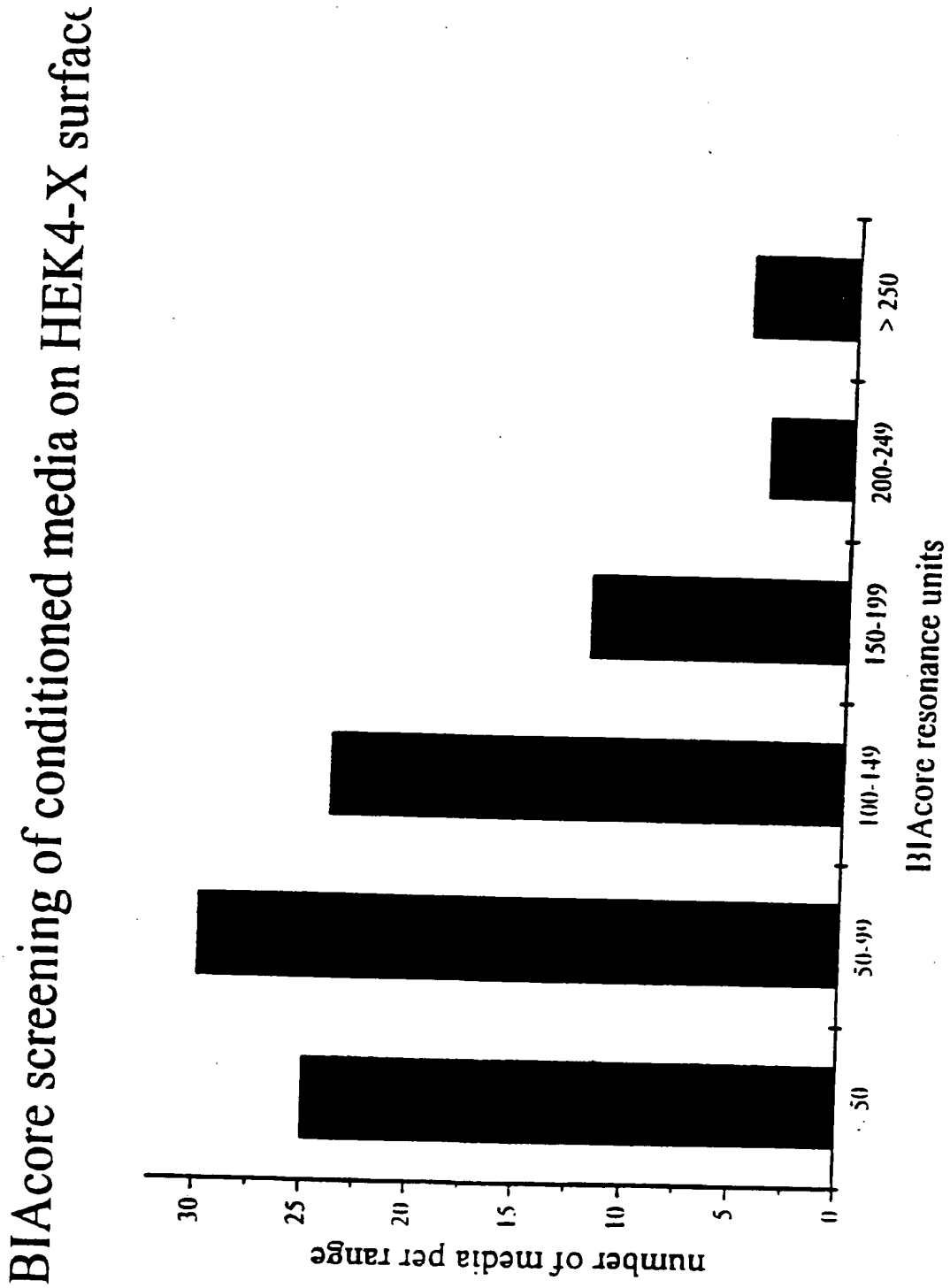
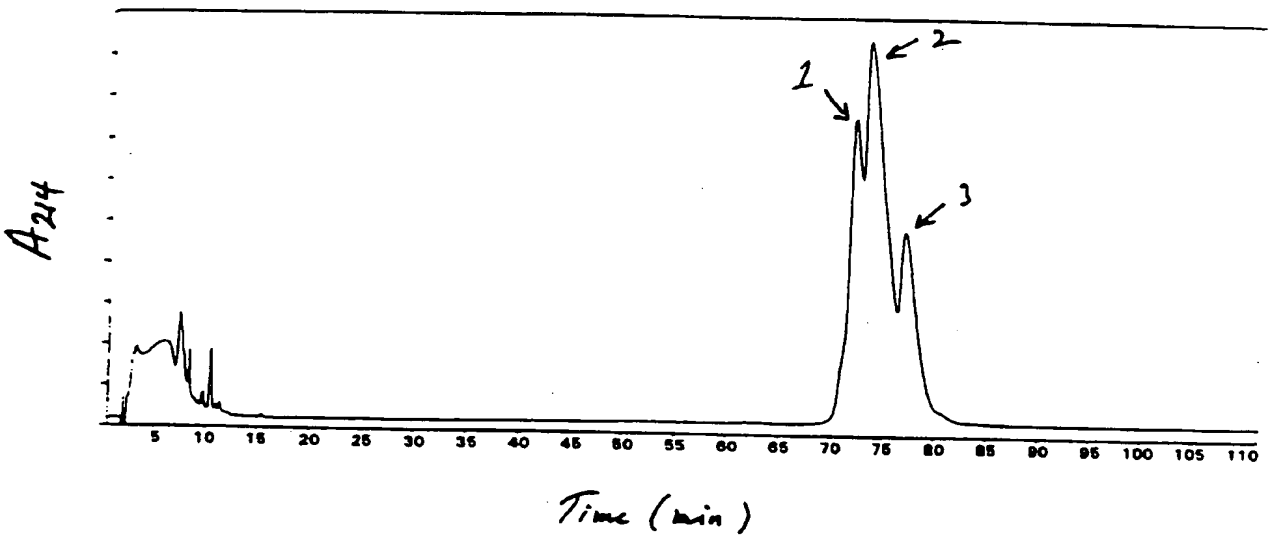

BIAcore screening of conditioned media on HEK4-X surfac
number of media per range
0
5
10
15
20
25
30
50
50-99
100-149
150-199
200-249
> 250
BIAcore resonance units

FIGURE 2

a)


A214
1
2
3
5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110
Time (min)

b)

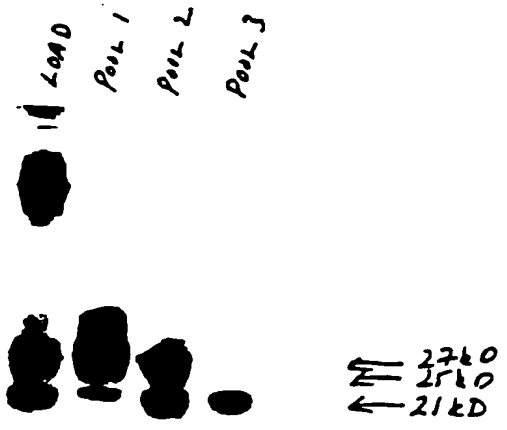

LOAD
POOL 1
POOL 2
POOL 3
27kD
25kD
21kD

FIGURE 3

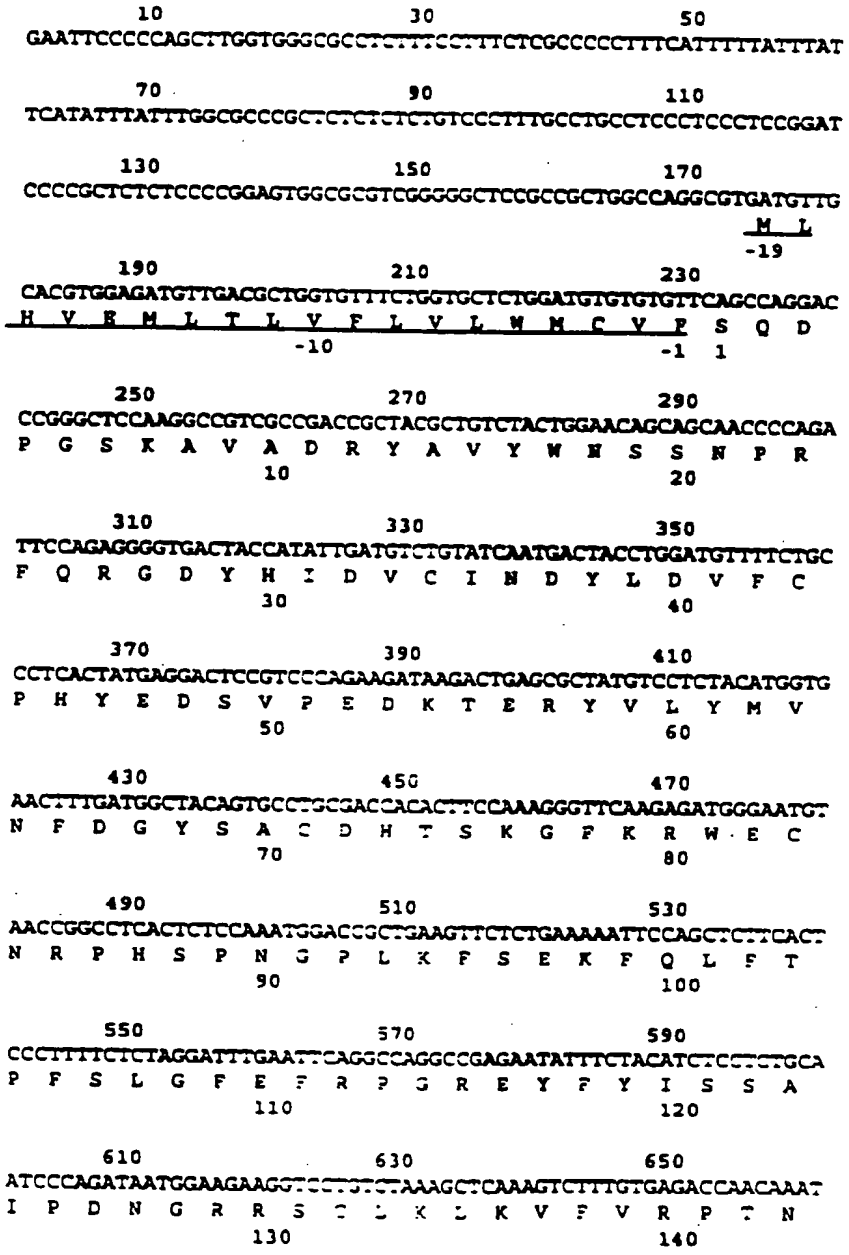

10 30 50
GAATTCCCCCAGCTTGGTGGGCGCCTCTTTCCTTTCTCGCCCCCTTTCATTTTTATTTAT
70 90 110
TCATATTTATTTGGCGCCCGCTCTCTCTCTGTCCCTTTGCCTGCCTCCCTCCCTCCGGAT
130 150 170
CCCCGCTCTCTCCCCCGGAGTGGCGCGTCGGGGGCTCCGCCGCTGGCCAGGCGTGATGTTG
M L
-19
190 210 230
CACGTGGAGATGTTGACGCTGGTGTTTCTGGTGCTCTGGATGTGTGTGTTCAGCCAGGAC
H V E M L T L V F L V L W M C V P S Q D
-10 -1 1
250 270 290
CCGGGCTCCAAGGCCGTCGCCGACCGCTACGCTGTCTACTGGAACAGCAGCAACCCCAGA
P G S K A V A D R Y A V Y W N S S N P R
10 20
310 330 350
TTCCAGAGGGGTGACTACCATATTGATGTCTGTATCAATGACTACCTGGATGTTTTCTGC
F Q R G D Y H I D V C I N D Y L D V F C
30 40
370 390 410
CCTCACTATGAGGACTCCGTCCCAGAAGATAAGACTGAGCGCTATGTCCTCTACATGGTG
P H Y E D S V P E D K T E R Y V L Y M V
50 60
430 450 470
AACTTTGATGGCTACAGTGCCTGCGACCACACTTCCAAAGGGTTCAAGAGATGGGAATGT
N F D G Y S A C D H T S K G F K R W E C
70 80
490 510 530
AACCGGCCTCACTCTCCAAATGGACCGCTGAAGTTCTCTGAAAAATTCCAGCTCTTCACT
N R P H S P N G P L K F S E K F Q L F T
90 100
550 570 590
CCCTTTTCTCTAGGATTTGAATTCAGGCCAGGCCGAGAATATTTCTACATCTCCTCTGCA
P F S L G F E F R P G R E Y F Y I S S A
110 120
610 630 650
ATCCCAGATAATGGAAGAAGGTCCTGTCTAAAGCTCAAAGTCTTTGTGAGACCAACAAAT
I P D N G R R S C L K L K V F V R P T N
130 140

FIGURE 3

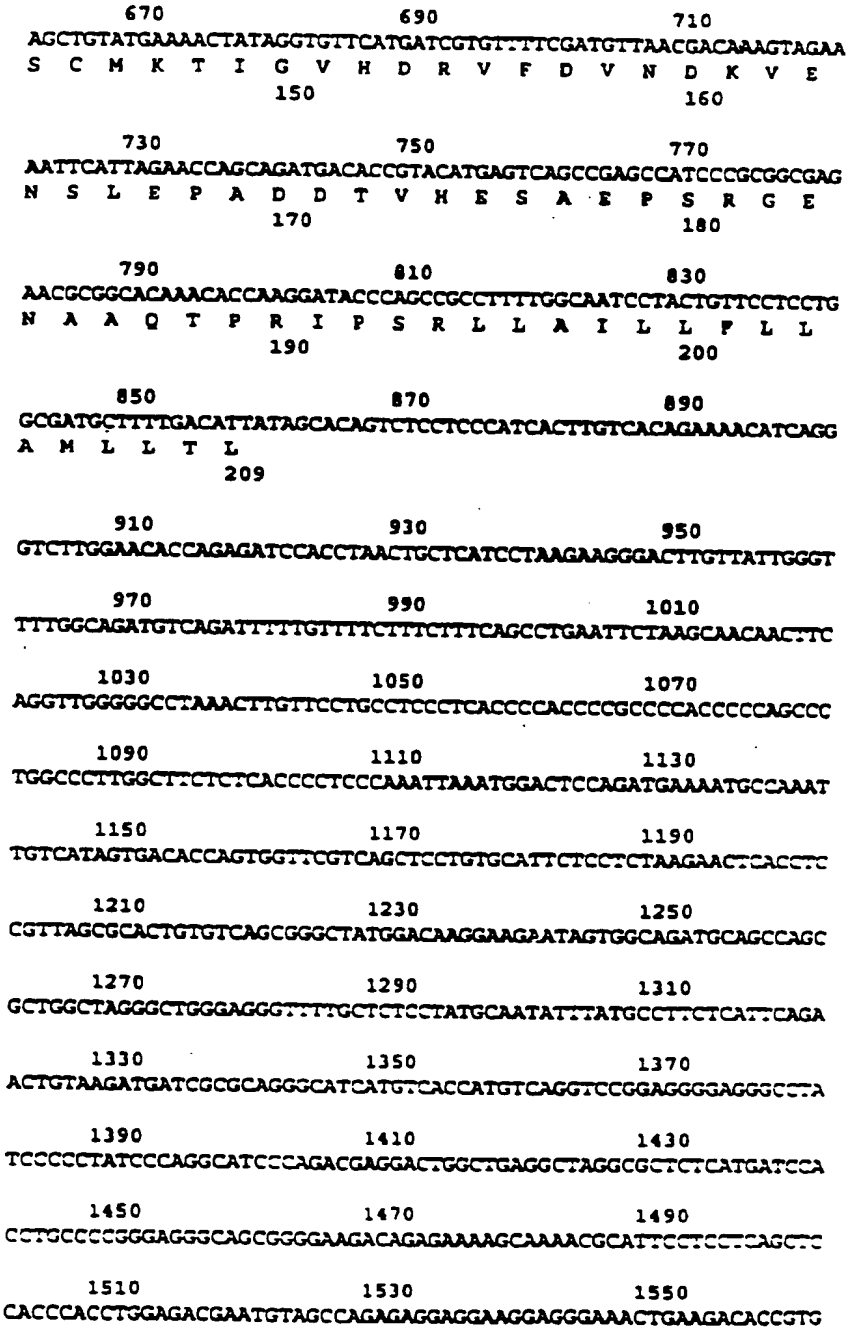

670
690
710
AGCTGTATGAAAACTATAGGTGTTCATGATCGTGTTTTCGATGTTAACGACAAAGTAGAA
S C M K T I G V H D R V F D V N D K V E
150
160
730
750
770
AATTCATTAGAACCAGCAGATGACACCGTACATGAGTCAGCCGAGCCATCCCGCGGCGAG
N S L E P A D D T V H E S A E P S R G E
170
180
790
810
830
AACGCGGCACAAACACCAAGGATACCCAGCCGCCTTTTGGCAATCCTACTGTTCCTCCTG
N A A Q T P R I P S R L L A I L L F L L
190
200
850
870
890
GCGATGCTTTTGACATTATAGCACAGTCTCCTCCCATCACTTGTCACAGAAAACATCAGG
A M L L T L
209
910
930
950
GTCTTGGAACACCAGAGATCCACCTAACTGCTCATCCTAAGAAGGGACTTGTTATTGGGT
970
990
1010
TTTGGCAGATGTCAGATTTTTGTTTTCTTTCTTTCAGCCTGAATTCTAAGCAACAACTTC
1030
1050
1070
AGGTTGGGGGCCTAAACTTGTTCCTGCCTCCCTCACCCCACCCCGCCCCACCCCCAGCCC
1090
1110
1130
TGGCCCTTGGCTTCTCTCACCCCTCCCAAATTAAATGGACTCCAGATGAAAATGCCAAAT
1150
1170
1190
TGTCATAGTGACACCAGTGGTTCGTCAGCTCCTGTGCATTCTCCTCTAAGAACTCACCTC
1210
1230
1250
CGTTAGCGCACTGTGTCAGCGGGCTATGGACAAGGAAGAATAGTGGCAGATGCAGCCAGC
1270
1290
1310
GCTGGCTAGGGCTGGGAGGGTTTTGCTCTCCTATGCAATATTTATGCCTTCTCATTCAGA
1330
1350
1370
ACTGTAAGATGATCGCGCAGGGCATCATGTCACCATGTCAGGTCCGGAGGGGAGGGCCTA
1390
1410
1430
TCCCCCTATCCCAGGCATCCCAGACGAGGACTGGCTGAGGCTAGGCGCTCTCATGATCCA
1450
1470
1490
CCTGCCCCGGGAGGGCAGCGGGGAAGACAGAGAAAAGCAAAACGCATTCCTCCTCAGCTC
1510
1530
1550
CACCCACCTGGAGACGAATGTAGCCAGAGAGGAGGAAGGAGGGAAACTGAAGACACCGTG

FIGURE 3

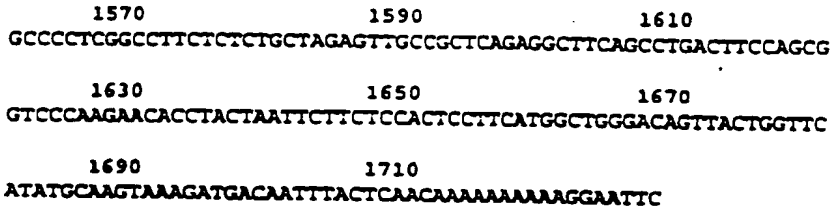
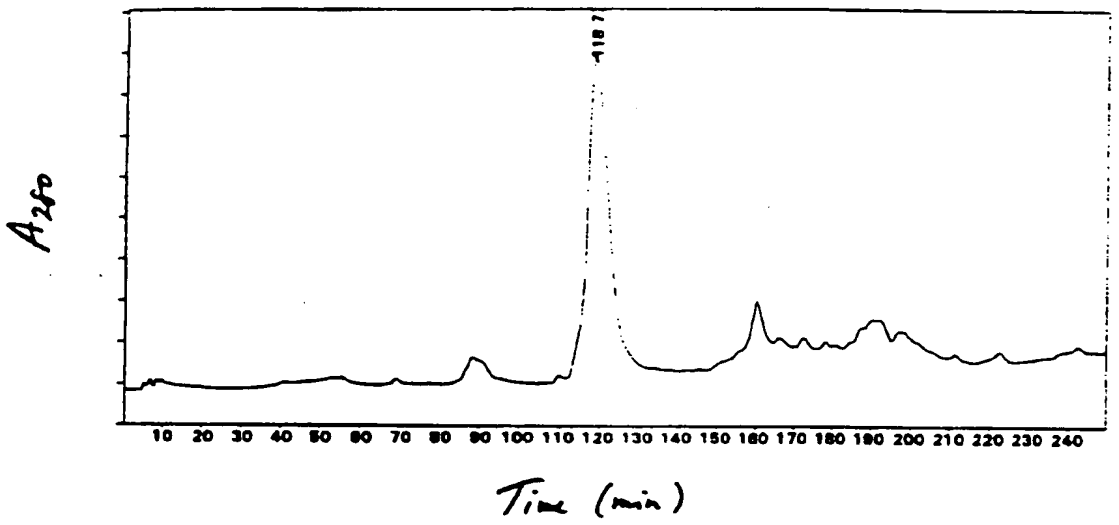
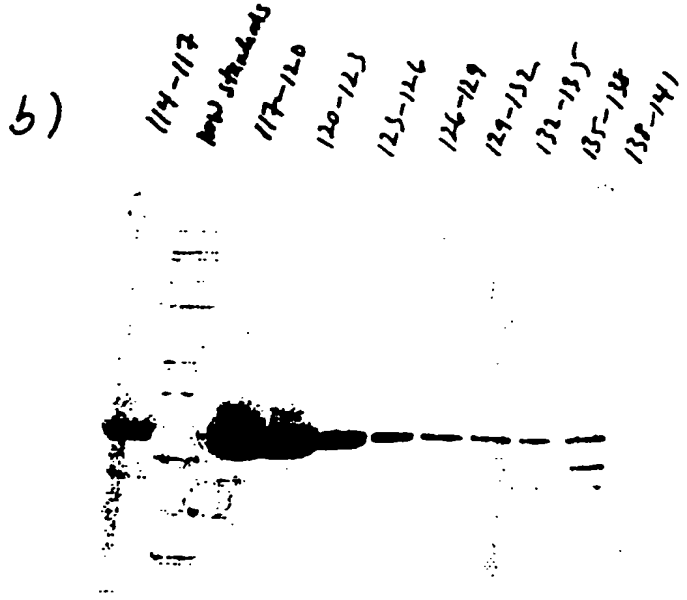

1570
1590
1610
GCCCCTCGGCCTTCTCTCTGCTAGAGTTGCCGCTCAGAGGCTTCAGCCTGACTTCCAGCG
1630
1650
1670
GTCCCAAGAACACCTACTAATTCTTCTCCACTCCTTCATGGCTGGGACAGTTACTGGTTC
1690
1710
ATATGCAAGTAAAGATGACAATTTACTCAACAAAAAAAAAAAGGAATTC

FIGURE 4

a)


A280
10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240
Time (min)

b)


114-117
MW standards
117-120
120-123
123-126
126-129
129-132
132-135
135-138
138-141

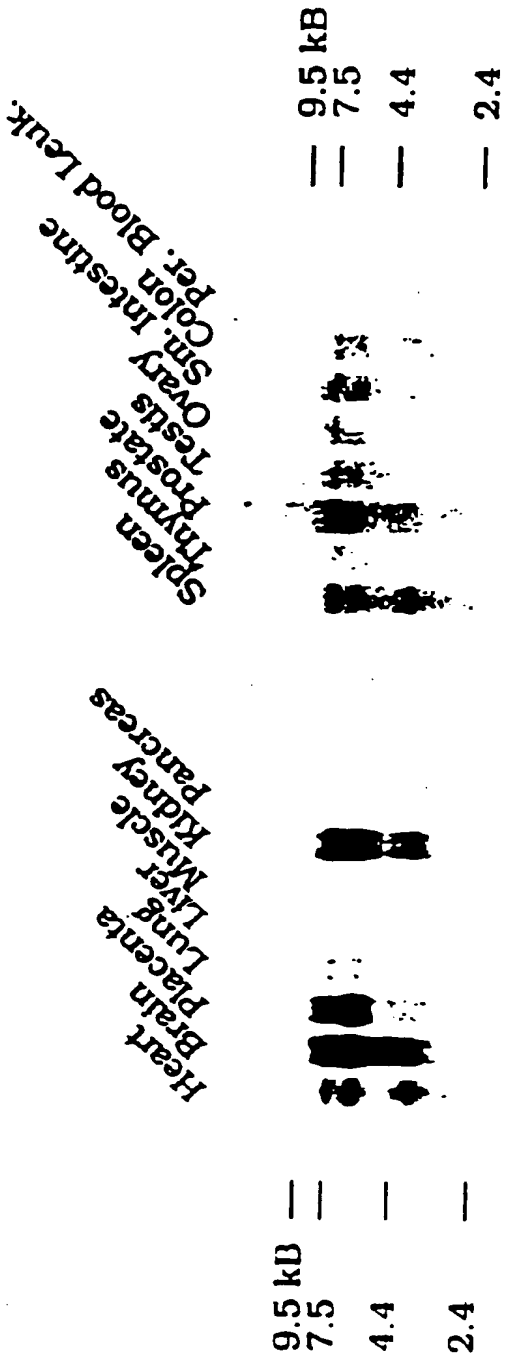

9.5 kB
7.5
4.4
2.4
Spleen
Thymus
Prostate
Testis
Ovary
Sm. Intestine
Colon
Per. Blood Leuk.
9.5 kB
7.5
4.4
2.4
Heart
Brain
Placenta
Lung
Liver
Muscle
Kidney
Pancreas

FIGURE 5

FIGURE 6

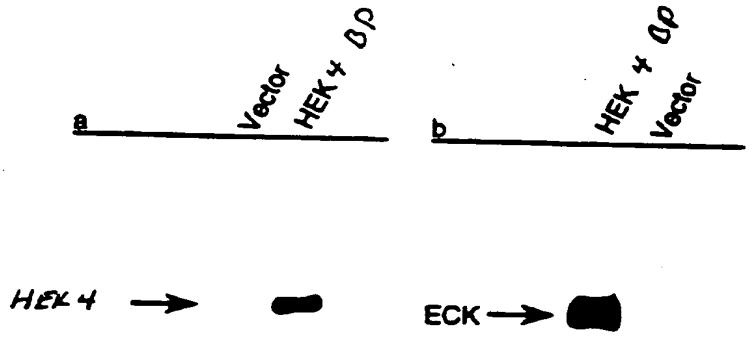


FIGURE 7

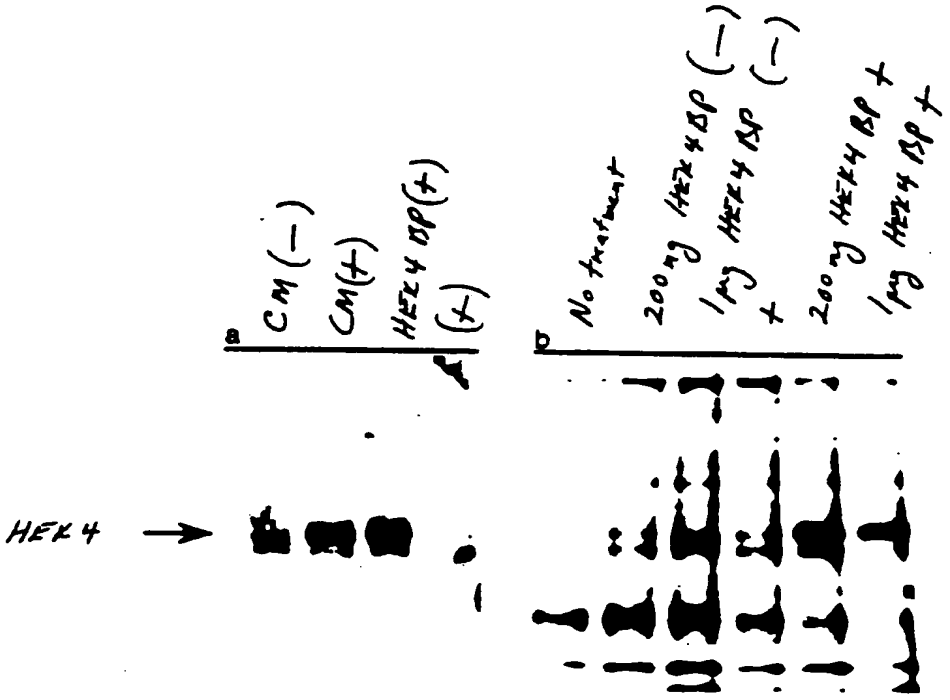
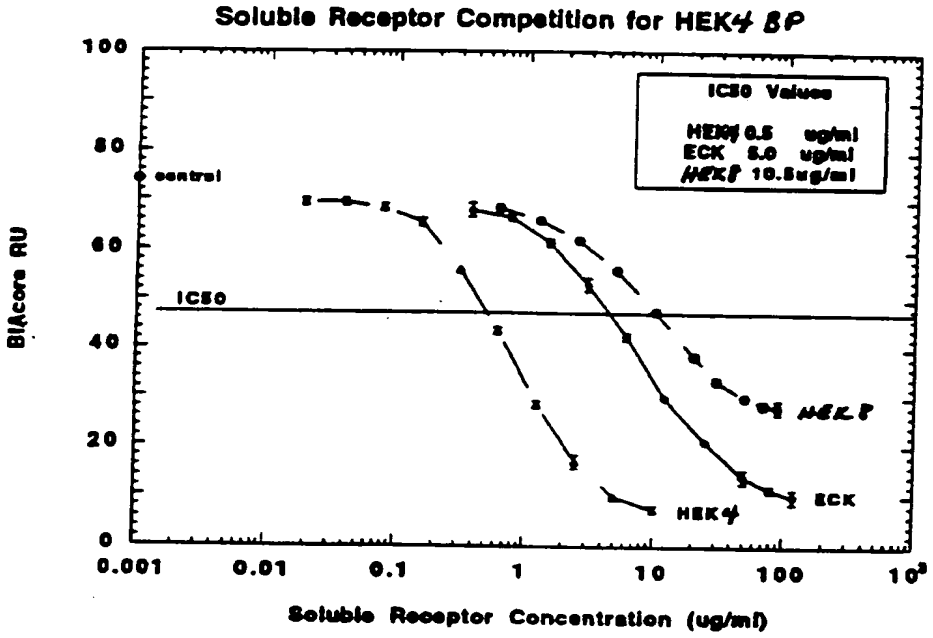

a
CM (−)
CM(+)
HEK4 BP(+)
(+)
b
No treatment
200ng HEK4 BP (−)
1mg HEK4 BP (−)
+
200ng HEK4 BP +
1mg HEK4 BP +
HEK4

FIGURE 8


Soluble Receptor Competition for HEK4 BP
IC50 Values
HEK4 0.5 ug/ml
ECK 5.0 ug/ml
HEK8 10.5ug/ml
control
IC50
HEK8
ECK
HEK4
BIAcore RU
Soluble Receptor Concentration (ug/ml)
100
80
60
40
20
0
0.001
0.01
0.1
1
10
100
10³

INTERNATIONAL SEARCH REPORT

Inter ·tional Application No
PC·/US 96/01079

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/47 C12N15/12 C07K16/18 C12N15/70 G01N33/68
C12Q1/68 A61K38/17 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N C12Q G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO,A,96 13518 (GENENTECH INC ;CARAS INGRID W (US); WINSLOW JOHN W (US)) 9 May 1996 see the whole document ---	1-35
P,X	NEURON, vol. 14, 5 May 1995, pages 973-981, XP002004532 J.W.WINSLOW E.A.: "Cloning of AL-1, aligand for an Eph-related TKR involved in axon bundle formation" see the whole document ---	1-12, 15-36
P,X	CELL, vol. 82, 11 August 1995, NA US, pages 359-370, XP002004533 U.DRESCHER E.A.: "In vitro guidance of retinal ganglion cell axons by RAGS..." see the whole document ---	1-8,11, 12,15-36
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
3 June 1996	12.06.96
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Authorized officer Groenendijk, M

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PC1/US 96/01079

C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP,A,0 597 503 (AMGEN INC) 18 May 1994 cited in the application see the whole document	1-36
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 89, March 1992, WASHINGTON US, pages 1611-1615, XP002004534 I.P.WICKS E.A.: "Molecular cloning of HEK..." cited in the application see the whole document	1-36

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/01079

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 28-33 encompass or are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition. ./..

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

Annex:

The peptides of the claims 1-7 are essentially only characterised by being "capable of binding at least one EPH-like receptor wherein the receptor is the HEK4 receptor". In the absence of any structural feature these peptides are considered to be ill-defined and the claims 1-7, 11-14 and the related claims 24-34 are considered to contravene Art.6 PCT, not allowing a complete search (Art.17(2)(a)(ii)PCT).
As far as the peptides of the present application are concerned the search has been directed to the peptides defined in claim 8 and fragments thereof.

Incomplete search:

Claims searched completely: 9-10, 15-23, 35, 36.
Claims searched incompletely: 1-7, 11-14, 24-34.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC./US 96/01079

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9613518	09-05-96	NONE		
EP-A-0597503	18-05-94	AU-B-	5599794	08-06-94
		CN-A-	1094446	02-11-94
		FI-A-	952328	10-07-95
		NO-A-	951861	11-07-95
		WO-A-	9411020	26-05-94